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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 27/30	A1	(11) International Publication Number: WO 90/13021 (43) International Publication Date: 1 November 1990 (01.11.90)

(21) International Application Number: PCT/GB90/00660

(22) International Filing Date: 27 April 1990 (27.04.90)

(30) Priority data:
8909613.5 27 April 1989 (27.04.89) GB

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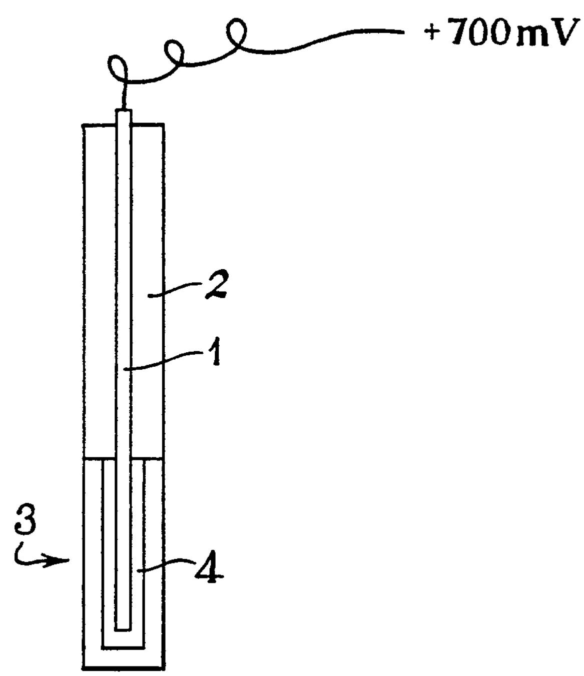
(81) Designated States: AT (European patent), BE (European + patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GLUCOSE-SENSING ELECTRODE



(57) Abstract

A glucose sensing electrode consists of a platinum wire (1) coated in synthetic hydrophilic polyurethane or polyhydroxyethylmethacrylate (4) containing glucose oxidase, this being itself coated in a mixture of hydrophobic polyurethane and hydrophilic polyurethane or hydrophilic polyhydroxyethylmethacrylate.

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GLUCOSE-SENSING ELECTRODE

FIELD OF THE INVENTION

This invention relates to a glucose-sensing electrode and to a glucose sensor using it. Such a sensor may find use in single measurements in, for example, samples of body fluids outside the body (e.g. blood, serum, plasma, urine), or, as an implantable sensor, in continuous measurement of glucose concentrations within the body.

DESCRIPTION OF PRIOR ART

Glucose sensors are probes which ideally would give a rapid and specific signal proportional to glucose concentration, without the need for added reagent. They have major potential applications in the management of diabetes mellitus, for home blood glucose monitoring (where a capillary blood sample obtained by finger-prick is spotted onto the sensor), for improved laboratory and ward glucose analysers which are currently expensive and bulky, and as implantable devices for continuous measurement of body glucose levels. The latter could be used as a read-out of diabetic "control" or to trigger an alarm when glucose concentrations fall to dangerously low or high levels (hypo- and hyperglycaemia). Alternatively, an implantable glucose sensor could be linked to an insulin infusion pump to provide automatic feedback control of insulin delivery (an artificial endocrine pancreas).

Other uses for glucose sensors in non-medical areas include monitoring or fermentation processes and food analysis.

Several technologies for glucose sensors have been described, including amperometric and potentiometric enzyme electrodes and optical approaches. For example, Diabetes Research Clinical Practice Supplement 1985 Vol 1 page S447 item 1162 states "Amperometric glucose sensors which are relatively oxygen insensitive have been constructed using 1,1'-dimethyl ferrocene (dicyclopentadienyl iron) to mediate glucose oxidase catalysed electron transfer between glucose and a carbon (graphite) base electrode. Several 1mm wide probe sensors, mounted on Plexiglass and suitable for in vivo implantation, were tested simultaneously

in vitro using a computer-assisted apparatus which poises the working electrode potential at +160 mV and records the current output. Electrodes dip-coated in 4% polyurethane were generally linear to at least 20 mmol/l glucose with a 95% response time of 30 sec. to 3 min." It was known, e.g. from Updike et al, Diabetes Care 1982 Vol 5 page 209 that "Oxygen is a hydrophobic gas and thus diffuses best through a hydrophobic membrane. On the other hand, glucose is a hydrophilic substance and diffuses best through a hydrophilic membrane", these being applied <u>between</u> the electrode and a layer of glucose oxidase. It is consequently taught by Updike to extend the linear range of glucose response by adding an outer hydrophobic layer to the glucose oxidase. Shichiri et al, The Lancet 20 November 1982 p 1129, describe a glucose sensor in which a platinum anode is coated with glucose oxidase immobilised in heparin and cellulose diacetate, an outer layer of polyurethane then being applied. The major problems with most sensors to date are unpredictable drift in output, sensitivity to changes in oxygen concentration at the sensing site and complex or intricate manufacturing procedures unsuitable for mass production.

<u>DESCRIPTION OF THE INVENTION</u>

According to the present invention, a glucose-sensing electrode comprises platinum (e.g. wire), or another base electrode capable of electrochemically oxidising hydrogen peroxide, coated in hydrophilic matter containing glucose oxidase, characterised in that said matter is a synthetic hydrophilic polymer. Preferably the polymer is applied in predominantly ethanolic aqueous solution, this having been found to be minimally damaging to the activity of the glucose oxidase enzyme compared with acetone, which has previously been used for coating enzyme-containing polymer. The polymer is preferably hydrophilic polyurethane and/or polyhydroxyethylmethacrylate.

Preferably, the coating of hydrophilic matter is itself coated in a mixture of hydrophobic matter and hydrophilic matter,

as we have found that this may extend the linearity of response of the electrode to glucose. This mixture may be polymeric; the hydrophobic component may comprise hydrophobic polyurethane, and the hydrophilic component is hydrophilic polyurethane and/or polyhydroxyethylmethacrylate.

The invention extends to a glucose sensor comprising the electrode set forth above.

The invention will now be described by way of example with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a glucose-sensing electrode according to the invention,

Figure 2 shows the current passed by two electrodes according to the invention at varying glucose concentrations in vitro,

Figure 3 shows the mean current at various times, and standard error of the mean, for a group of five electrodes according to the invention operated in vitro for 24 hours, this to illustrate the stability of the electrodes,

Figure 4 shows the in vivo response of an electrode according to the invention compared with the blood glucose level analysed conventionally, and

Figure 5 shows the effect of varying oxygen concentration at fixed glucose concentration on the output of electrodes according to the invention.

EXAMPLE OF THE INVENTION

Turning to Figure 1, the construction of a glucose-sensing electrode according to the invention is shown.

Platinum wire 1 is dipped in insulating varnish 2 and baked at 80°C for 2hr in an oven. After cooling, an approximately 5mm length 3 is scraped from one end of the wire for application of sensing material 4. This material consists of a mixture of (a) glucose oxidase enzyme dissolved in water and (b) either polyhydroxyethylmethacrylate polymer or hydrophilic polyurethane in 75% ethanol/25% water. The concentration of (a) is 1000 IU/ml, and of (b) is 12.5% w/v, and the volume ratio of (a):(b)

is 1:3. Alternatively, (a) as a step in making the mixture can be omitted, and dry glucose oxidase mixed with the hydrophilic polymer in the ethanol/water to a total concentration of say 5000 IU/ml glucose oxidase. After dip-coating in the enzyme/polymer mixture, the wire is air-dried at room temperature for 20 min. This is simpler than covalent enzyme-attachment procedures using substances such as glutaraldehyde.

An outer membrane application material consists of a mixture of (a) hydrophobic polyurethane in tetrahydrofuran optionally with ethanol (concentration 10%) and (b) either the hydrophilic polymer polyhydroxyethylmethacrylate in 75% ethanol or hydrophilic polyurethane in tetrahydrofuran, concentration 10%, ratio a:b 3:1. After dip-coating in this material, the electrode is air dried for 20 min at room temperature.

In vitro, a pseudoreference (combined reference and auxiliary electrode) consisting of silver/silver chloride adjacent to the working electrode described, or in vivo a silver/silver chloride electrocardiogram electrode on the skin above the implanted working electrode, makes a glucose sensor according to the invention. The working electrode is operated in amperometric mode at a fixed 700mV.

Before routine use, the sensor may be preconditioned by operating at +700mV in 5mmol/l buffered glucose solution at pH 7.4 for 18 hr at 37°C.

Fig. 2 shows _in vitro_ calibration curves for two glucose sensors, which demonstrate high current outputs which increase linearly to at least 20 mmol/l glucose. These sensors were as described for Figure 1, with 10% polyhydroxyethyl-methacrylate/glucose oxidase inner membrane and 75% hydrophobic polyurethane/25% hydrophilic polyhydroxyethyl-methacrylate outer membrane.

Fig. 3 shows the mean current ± standard error of the mean for a group of 5 glucose sensors operated _in vitro_ at 37°C for 24hr in 5mmol/l glucose solution, which demonstrates the excellent stability of the devices.

Fig. 4 shows the in vivo response of an electrode according to the invention compared with the blood glucose level analysed conventionally. This demonstrates the possibility of the use of the glucose sensor as an implantable monitor of glycaemic control in man. A sensor was implanted in the subcutaneous tissue of the forearm of a normal volunteer subject. The potential was set at +700mV using a potentiostat and a surface electrocardiogram electrode used as a reference in a two-electrode configuration. At time 0, 75g of glucose was administered orally and blood glucose concentrations measured conventionally using a Yellow Springs Instruments analyser, a bench-top instrument for in vitro blood sample analysis, having an immobilised glucose oxidase membrane and operating by detection of the produced hydrogen peroxide. Electrode current readings were calibrated by assuming that the initial current value is equivalent to the initial blood glucose level. The graph demonstrates that tissue glucose levels measured by the sensor according to the invention follow blood glucose levels (measured conventionally) with little delay, though of lesser magnitude. The implanted sensor can thus be used to monitor changes in blood glucose levels.

Fig. 5 shows an example of the mean current output of 5 glucose sensors operated in a fixed concentration of buffered glucose solution _in vitro_ at 20 kPa p[O_2] and at 1 kPa p[O_2] (viz, one-fifth of an atmosphere of oxygen (as normal) and 0.01 atmosphere respectively) which demonstrates that the sensors are not significantly affected by changing concentrations of oxygen. These sensors did not have the outer mixed-hydrophobic-hydrophilic membrane.

Thus, such a glucose sensor could be used as an implantable device:

(a) to give a continuous measure of glycaemic control.

(b) to detect hypoglycaemia and sound an alarm at a pre-set glucose level

(c) to detect hyperglycaemia and sound an alarm at a pre-set glucose level

(d) to be linked to a portable or implanted insulin infusion pump with feedback control of insulin delivery according to the prevailing glucose levels and also as a non-implanted device:

(e) to provide a small, hand-held device for monitoring glucose levels in blood, serum or plasma samples or in other body fluids. This might be configured as a pen-type device with digital readout and incorporating the sensor at one end.

(f) as a small bench-top glucose analyser for laboratory, ward, bed-side, office or other use, and

(g) to form part of a multi-analyser system (glucose plus other analytes).

CLAIMS

1. A glucose-sensing electrode, comprising an inert conductor coated in hydrophilic matter containing glucose oxidase, characterised in that said matter is a synthetic hydrophilic polymer.

2. An electrode according to Claim 1, wherein said polymer is applied in predominantly ethanolic and/or aqueous solution.

3. An electrode according to Claim 1, wherein the polymer is hydrophilic polyurethane and/or polyhydroxyethylmethacrylate.

4. An electrode according to Claim 2 wherein the polymer is hydrophilic polyurethane and/or polyhydroxyethylmethacrylate.

5. An electrode according to Claim 1, 2, 3 or 4 wherein the coating of hydrophilic matter is iself coated in a mixture of hydrophobic matter and hydrophilic matter.

6. An electrode according to Claim 5, wherein the mixture is polymeric.

7. An electrode according to Claim 5, wherein the hydrophobic matter comprises hydrophobic polyurethane.

8. An electrode according to Claim 6 wherein the hydrophobic matter comprises hydrophobic polyurethane.

9. An electrode according to Claim 6, 7 or 8 wherein the hydrophilic matter of said mixture comprises hydrophilic polyurethane and/or polyhydroxyethylmethacrylate.

10. An electrode according to Claim 1, 2, 3, 4, 6, 7 or 8 wherein the inert conductor is platinum.

11. An electrode according to Claim 5 wherein the inert conductor is platinum.

12. An electrode according to Claim 9 wherein the inert conductor is platinum.

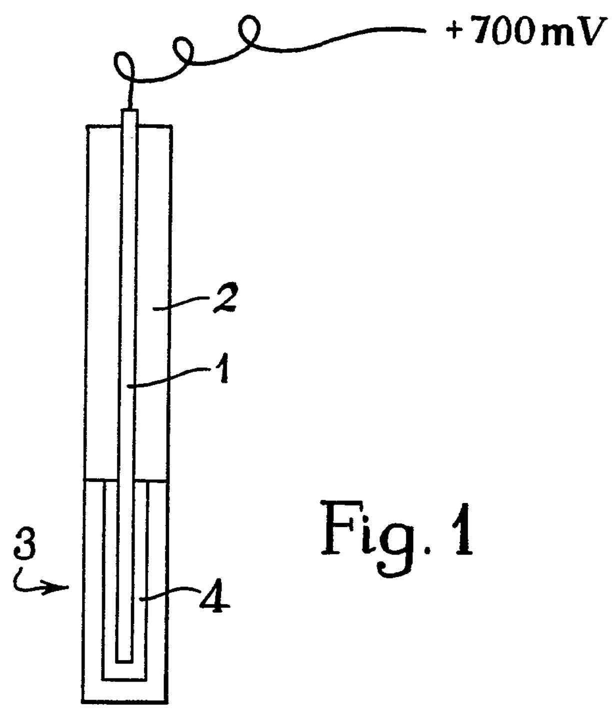
+700mV
2
1
3
4

Fig. 1

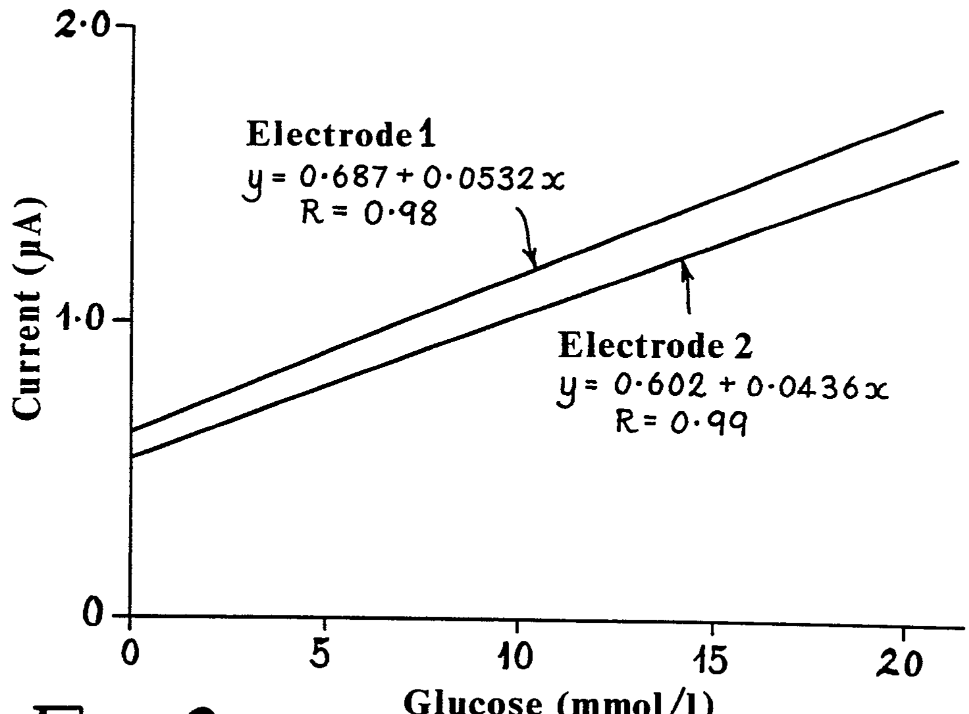
Current (μA)
2·0
1·0
0
Electrode 1
y = 0·687 + 0·0532 x
R = 0·98
Electrode 2
y = 0·602 + 0·0436 x
R = 0·99
0
5
10
15
20
Glucose (mmol/l)

Fig. 2

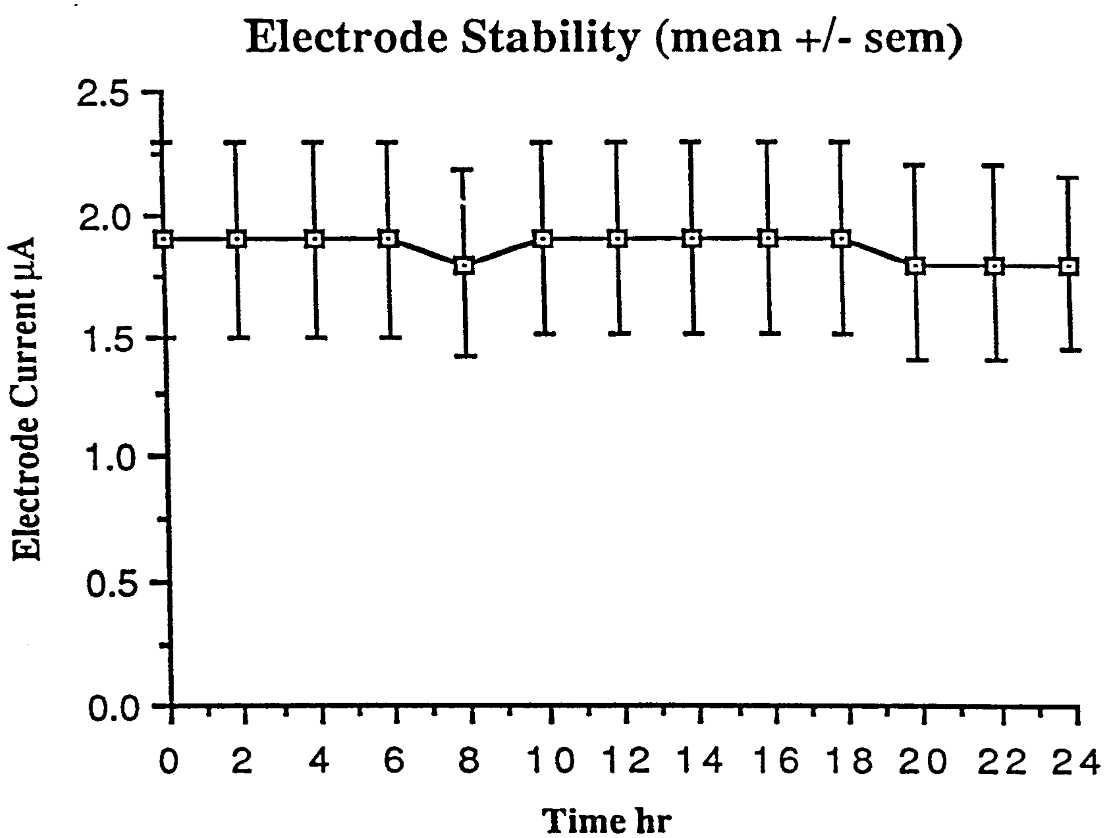
Electrode Stability (mean +/- sem)
Electrode Current µA
2.5
2.0
1.5
1.0
0.5
0.0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time hr

Fig. 3

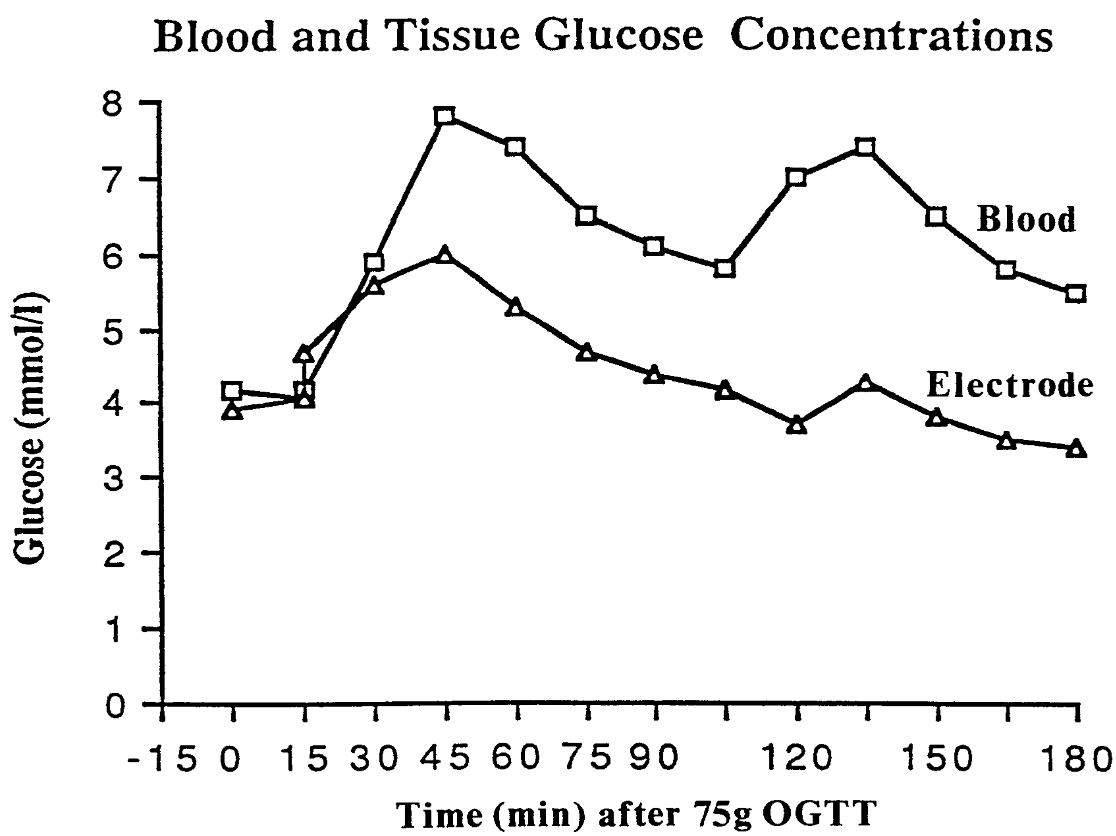
Blood and Tissue Glucose Concentrations
Glucose (mmol/l)
8
7
6
5
4
3
2
1
0
Blood
Electrode
-15 0 15 30 45 60 75 90 120 150 180
Time (min) after 75g OGTT

Fig.4

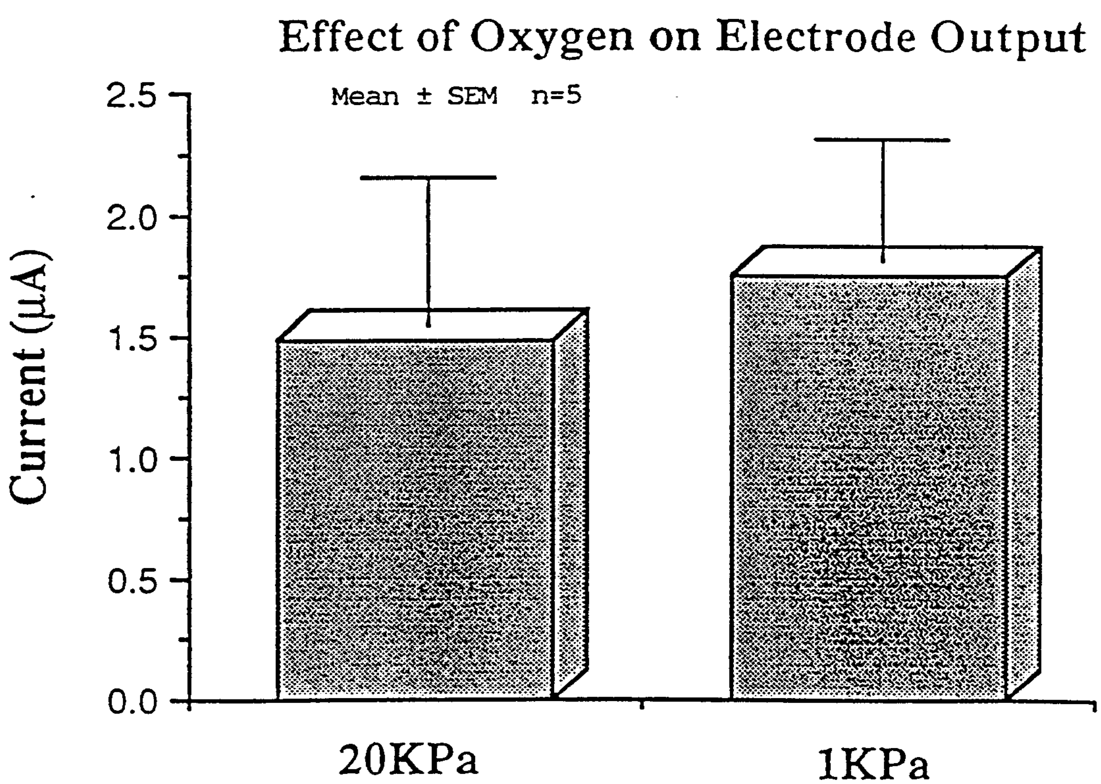
Effect of Oxygen on Electrode Output
Mean ± SEM n=5
Current (μA)
2.5
2.0
1.5
1.0
0.5
0.0
20KPa
1KPa

Fig. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00660

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N27/30

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12M ; G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X,P	WO,A,8907139 (NOVO INDUSTRI A/S) 10 August 1989 see claims 1-3, 8, 22, 23; figure 1 ---	1--6, 9, 11, 12
X	DE,A,3824258 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 16 February 1989 see column 4, lines 1 - 22; claims 4, 7 ---	1-6
X	DE,A,2903216 (TOYO BOSEKI K.K.) 02 August 1979 see page 15, line 25 - page 17, line 3 ---	1-6
X	EP,A,216467 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 01 April 1987 see claim 8 ---	1-6

-/--

° Special categories of cited documents : [10]

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 AUGUST 1990	11. 09. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	EPAILLARD P.J.H. P. Epaillard

1

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	CHEMICAL ABSTRACTS, vol. 104, 1986 Columbus, Ohio, USA omicron scientific ltd: "preparation of an enzyme membrane for use in biosensors" page 287; left-hand column; ref. no. 48317B see abstract ---	1-6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9000660
SA 36677

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 14/08/90
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8907139	10-08-89	AU-A-	3064689	25-08-89
DE-A-3824258	16-02-89	None		
DE-A-2903216	02-08-79	JP-A,B	54102193	11-08-79
		US-A-	4240889	23-12-80
EP-A-216467	01-04-87	AU-B-	591150	30-11-89
		AU-A-	6038686	26-03-87
		CA-A-	1247700	27-12-88
		JP-A-	62073150	03-04-87
		US-A-	4890620	02-01-90

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